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Towards functional characterization of CAZy family GT77 Glycosyltransferases

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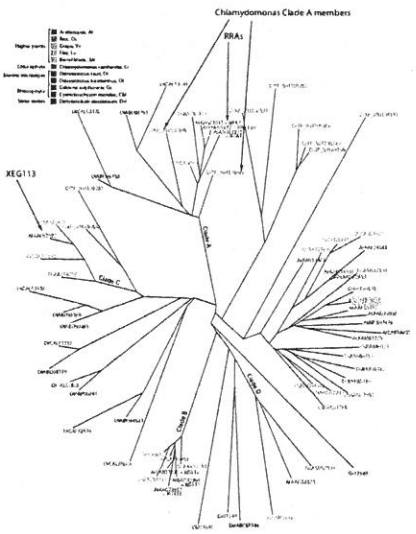
Background

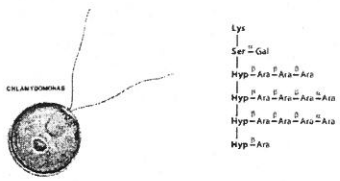
Two GT-family-77 Arabidopsis mutants, *rra-1* and *rra-2*, were shown to have a reduced residual arabinose phenotype in a fraction that is not readily released from the cell wall. Involvement of the corresponding wild type genes RRA1 and RRA2 in arabinosylation of extensins was suggested (Egelund et al. 2007, Plant Mol. Biol. 64:439-451). Recently, a xyloglucanase based screen on Arabidopsis T-DNA-mutants identified a mutant, *xeg113*, where the extensin component of the cell wall was under-arabinosylated (Gille et al. 2009, P.N.A.S., in press). Interestingly XEG113 (At2g35610) is also classified in GT-family-77.



Two activities are known in family GT77, the rhamnogalacturonan-II β-1,3-xylosyltransferases from arabidopsis (clade B) and the α-1,3-galactosyltransferase from the slime mold *Dictyostelium*. These activities, however, cannot readily account for the observed reduced arabinose phenotype in the knock-out mutants.

How to account for the reduced amount of arabinose in the walls of *rra* and *xeg113* mutants

GT77 is an old family where some clades, like clade A and -C, have remained stable since the divergence of the green plant lineage.



Chlamydomonas' cell wall is not built of polysaccharides. Rather, it is made of extensin-like proteins rich in arabinose and to a lesser extent galactose. (Structure of higher plant extensin (right), Hyp=hydroxyproline)

The similarity of RRA1-3 and XEG113 to glycosyltransferases from *Chlamydomonas reinhardtii* has prompted us to propose that the reduced arabinose phenotype of the mutants shall be explained in terms of compromised extensin arabinosylation.

The following scenarios are considered:

The RRAs and XEG113 are:

1. not arabinosyltransferases. The reduced arabinose phenotype is a pleiotropic effect.

2. inverting arabinosyltransferases (contrary to what is expected of GT77s) and thus create α-arabinans.

3. retaining UDP-*araf* : extensin Hyp β-AraTs and hence add the innermost Araf to extensin Hyp residues.

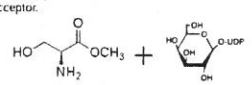
4. retaining UDP-*araf* : extensin $(Ara)_{1-2}$ β-AraTs and hence transfer the second or third arabinofuranosyl residue of extensin arabinans.

The assays of heterologously expressed RRA1-3 and XEG113

1. RRAs and XEG113 are not arabinosyltransferases.

General tests can obviously not be implemented. One particular derived effect can be tested: If extensin arabinosylation depends on prior galactosylation of the extensin serine residues sitting next to the HYPs, then knock-out of an UDP-Gal : extensin Ser α -GalT could result in reduced arabinosylaion.

The assay performed used UDP-^{14}C-Gal as donor and serine methyl ester as acceptor.

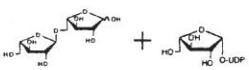


No incorporation, so RRA1-3 and XEG113 are not Ser α-GalTs.

2. RRAs and XEG113 are inverting arabinosyltransferases (contrary to what is expected of GT77s) and thus create α-arabinans.

Arabinobiose (α-arabinosyl-1,5-arabinose) was used as acceptor, and UDP-*araf* was used as donor.

Acceptor and potential product = arabinotriose separated by paper chromatography before mass spectrometry. Residual arabinobiose could be seen, but no triose was formed.



Conclusion: If RRA1-3 and XEG113 are arabinosyltransferases, they are probably retaining as expected of GT77s.

3. RRA1-3 and XEG113 are retaining UDP-*araf* : extensin HYP β-AraTs and hence add the innermost Araf to extensin Hyp residues.

The assay was performed with a synthetic polypeptide kindly provided by José Estévez and Chris Somerville as acceptor and UDP-araf as donor

5-carboxyfluorescein-**ASOOOSOOOSOOOAHHHHHH**, O=Hyp

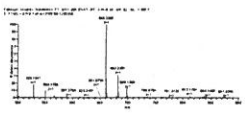
RRA1 & 2: No incorporation, so RRA1 & RRA2 are proberbly not the innermost Hyp β-AraFTs.

RRA3 & XEG113: Waiting for MS analysis...

4. RRAs and XEG113 are retaining UDP-*araf* : extensin $(Ara)_n$ β-AraTs and hence attach the second, third arabinofuranosyl residue of extensin arabinans.

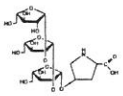
Assay: Extensin from late exponential stage BY2 suspension cells was extracted by 'in wall' $Ba(OH)_2$ mediated hydrolysis and purified on Superdex Peptide, and finally fractionated on Dionex Carbopack PA1.

Single Hyp-residues with arabinan side chains were isloated. As anticipated the far most aboundant species was Hyp-(Ara)4, suggesting an α-linked outermost arabinofuranose.



Hyp-(Ara)4: MW (H)+H$^+$ (zwitterion) 660.2

The Hyp-(Ara)4 fraction were treated with an α linkage specific arabinofuranosidase in order to generate Hyp-β-linked arabinotriose



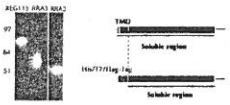
Verification of Hyp-β-linked arabinotriose and GT assays containing this acceptor are

Waiting for MS analysis...

Heterologous expression of GT77 members

RRA1, RRA2, RRA3 (At1g19360) and XEG113 were expressed as secreted soluble enzymes in *Pichia pastoris* (RRA3) or in insect Sf9 cells:

	MW (kDa)
RRA2HT7FS:	48
RRA3HT7FS:	49
Xeg113HT7FS:	73.6



Anti Flag Western of crude media

The four enzymes were His-tag purified and desalted prior to incubation in assays.

References

Egelund J, Obel N, Ulvskov P, Geshi N, Pauly M, Bacic A, Petersen BL (2007) Molecular characterization of two Arabidopsis thaliana glycosyltransferase mutants, *rra1* and *rra2*, which have a reduced residual arabinose content in a polymer tightly associated with the cellulosic wall residue. PMB 64: 439-451

Sascha Gille, Ulrike Haense, Mark Ziemann, Markus Pauly (2009) Identification of plant cell wall mutants by means of a forward chemical genetic approach using hydrolases. Proc. Acad. Sci., USA, in Press